THE STABILIZED ADDICTION STATE AND THE EEG

PRELIMINARY OBSERVATIONS

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THE STABILIZED ADDICTION STATE AND THE EEG:

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INTRODUCTION

The state of addiction to morphine and other opiates, barbiturates and alcohol has long been recognized as being associated with behavioral and psychiatric disturbances.(1) The mechanism of the pathogenesis of these disturbances remains obscure, as does the understanding of the biophysical and biochemical phenomena underlying the more highly reproducible systemic effects of these drugs, i.e., drug tolerance. Three possibilities suggest themselves:

(A) That the behavioral and psychiatric disturbances are due to a direct, chronic effect of the drug on the central nervous system. Ignoring for the moment that administration of morphine or other narcotics is accompanied by tachyphylaxis and tolerance, it would be difficult to reject the argument that a drug which exerts such profound neurological effects after administration (hypnosis, hypesthesia, euphoria) should continue to exert some effect as long as it is administered. If we accept the phenomena of tachyphylaxis and tolerance as empirically observed, we are forced to the conclusion that the chronic effects of such a drug are entirely distinct from its acute effects, and thus we have very little basis for ascribing the changes observed in narcotic addiction to a primary effect of the drug itself. On the other hand, the presence of dependence to a particular addicting drug would seem to indicate that at least some recognizable and unique portion of the drug is retained in the nervous system during the addiction state, and because of the organism's ability to "recognize" its particular addicting agent, withdrawal symptoms will not be ameliorated by any other agent than one which is structurally related to the drug which has been administered chronically.

(B) That they are due to the effects of changes in the concentrations of intra- or extracellular substances in the central nervous system, or due to the liberation of new substances, this change having been brought about as a secondary effect of the chronic administration of the drug. It is certainly true that narcotic addiction is accompanied by widespread systemic effects. Nevertheless, consistent biochemical alterations in central nervous system or other tissue has not yet been demonstrated as a consequence of drug addiction.(3) Moreover, if the observable stigmata of narcotic addiction were not the result of a primary effect of the narcotic itself, then treatment of withdrawal symptoms in the acute phase should be directed toward the secondary alterations which had occurred as a result of the use of the drug. However, though symptomatic treatment of acute narcotic withdrawal is at present the only medical means of coping with the problem of addiction per se, the relief of these symptoms by the re-administration of the narcotic itself is much more dramatic.

(C) That physical or biochemical effects of the drug on the central nervous system are minimal during a period of "stabilized" addiction; the behavioral and psychiatric alterations are largely the result of environmental and social factors which have changed because of the addict's dependence upon the drug. The "stabilized addiction" state can be defined as that period during addiction when the amount of drug administered is sufficient to prevent withdrawal symptoms, but not great enough to elicit the acute effects associated with the initial administration of the addicting agent. Such a state should be relatively easy to achieve in the experimental situation; the amount of drug necessary to reproduce the effects of the initial dose increases geometrically with each succeeding dose, while the amount necessary to prevent withdrawal symptoms rises much more slowly, if at all.

Several cases of stabilized addiction states in humans unaccompanied by overt psychiatric disturbances, maintained over long periods, have been recorded.(2) It is true that study of the stabilized addiction state may not yield information of value concerning that type of drug addiction most often seen clinically, where the effects sought for by the addict are the acute effects (euphoria, etc.) which depend upon an ever-increasing dose, rather than simply the prevention of withdrawal symptoms. Nevertheless it would be of value to observe a reproducible, measurable alteration in some biophysical or biochemical parameter that occurs with chronic administration of an addicting drug, even in the absence of behavioral disturbances; and the stabilized addiction state can then be readily seen to be more easily evaluable experimentally, than the transient parameters which are associated with early addiction and withdrawal.

Narcotic addiction rarely presents clinically as an isolated disturbance; thus, the setting up of control groups to differentiate between the effects of narcotic addiction and unrelated, but coincident disturbances (alcoholism, dietary deficiences, etc.) may prove to be an impossible task. For this reason, it is preferable to use animal subjects when only the physiological aspects of narcotic addiction are to be investigated, since the social environment of the animal can be rigidly controlled by the experimenter. The use of experimental animals, the laboratory rat in particular, is attended, however, by many possible sources of error. Among these are:

(A) The rat, and lower mammals in general, are much less susceptible to the acute effects of a narcotic such as morphine than is the human, as far as both the production of behavioral effects and toxicity are concerned.(4) Also, the acute effects produced are different; in man, depression of the

central nervous system is the predominant observable effect, while lower mammals may often exhibit symptoms of excitation of some nervous areas. In rodents, a marked state of somnolence accompanied by increased reflex irritability may occur.

(B) Whether or not a rat can be addicted to a narcotic by repeated, chronic administration of the drug has not been firmly established; moreover, it is debatable whether the syndromes described in rats which are attributed to narcotic addiction and withdrawal can be considered analogous to those which occur in humans. One author has characterized addiction in rats by hyperexcitability, and withdrawal by markedly decreased locomotion,[5] accompanied by a lowered threshold for the occurrence of seizures. These parameters are difficult to quantify accurately. Even if they were quantified, however, they still do not closely correspond with the more definitive syndromes observed in man.

The electroencephalogram has been noted to be a sensitive and reproducible index of central nervous system responses to a wide variety of substances.[7] In particular, it is valuable in demonstrating the possible existence of an organic basis for psychiatric and behavioral disturbances.[6] A study of the EEG responses to addicting drugs in lower mammals has not heretofore been carried out, and could provide an effective background for the evaluation of drug effects.

Proper performance of such a study should provide for the following:

(a) A suitable method of recording electroencephalograms from small mammals such as the rat over a long period of time, i.e., several months, in an atraumatic and reproducible manner. For an animal such as the laboratory rat, variations in positioning of the electrodes from recording to recording would undoubtedly cause variation in the reproducibility of the

results. Accordingly, it would be most satisfactory if chronically implanted electrodes were used for recording.

(b) A suitable baseline for normal, resting EEG tracings. The prolonged handling of experimental animals such as the rat during the recording procedure, together with the stresses imposed by anesthesia and an operative procedure (in the acute animal) may certainly be reflected in the EEG tracing. Continuous restraint of the animal to minimize muscle and movement artefact may also constitute a significant stress. It has been found that even minimal handling of a laboratory rat will significantly raise its serum corticosterone level within two minutes, while the return to normal levels is greatly prolonged.(8) The most suitable recording technique, then, would be one that did not involve handling the animal at the time the tracing was obtained. This is best accomplished by chronic implantation.

(c) A set of satisfactory criteria of drug addiction, preferably criteria that correspond clinically to those used for diagnosing drug addiction in humans.

(d) A suitable method of classifying and quantifying the EEG responses obtained in the experimental situation.

TECHNIQUES

I. Skull Electrodes

These were prepared in the following manner: One end of a thin gauge (3-4 mils) teflon-insulated copper wire was soldered to the head of a stainless steel jeweler's watch-case screw, (the length of the screw was about 3/32 inch, and the outside diameter of the thread was approximately 22/1000 inch) using phosphoric acid as the soldering flux. The wire was fixed to the screw so that the solder did not flow either under the head of the screw, or into the slot. The other end of the wire was soldered into the center of a segment of 20 or 21 gauge stainless steel hypodermic tubing, about 1.5 centimeters in length. The total length of the wire segment was between 0.75 and 1.0 centimeters. The jeweler's screws had a two-fold purpose; firstly, they were to serve as low-resistance skull electrodes, which would extend through the parietal bone, only as far as the dura, without dural penetration or penetration of cerebral substance. Secondly, the engagement of the thread with the animal's parietal bone would insure that the electrode would remain fixed in its position, and in addition, would provide firm support for the framework of hypodermic tubing and connecting wires.

To accommodate these screws, small holes (18/1000 inch in diameter) were drilled in the parietal bones of the experimental animals. In order to insure accurate and tight fit of the screws, the following apparatus was devised: The skull-gripping device from a standard rat stereotaxic instrument was mounted upon a horizontal pair of polished aluminum rods, so that this portion of the apparatus could slide forward and backward along the axis of the rods. Above this arrangement, the handpiece of a flexible-shaft dental drill was mounted vertically into a steel framework, which

permitted side-to-side motion of the drill, while its vertical orientation remained unchanged. Fine motion along the vertical axis of the handpiece was controlled by a screw-thread adjustment on the drill mounting.

The accurate size, positioning, and orientation of these holes was thus reproducibly insured.

II. Implantation of Electrodes

Each experimental animal was anesthetized with Nembutal, 40 mg./kg. and Atropine, 0.01 mg./kg., and placed in the apparatus. A midline incision was made, beginning at a point just behind the eyes of the animal, and was continued to about one centimeter posterior to the external ears. All layers superficial to the periosteum were then retracted laterally, and tension was placed upon the incision both anteriorly and posteriorly. This assured adequate exposure for the remainder of the procedure. At this point, some bleeding was usually encountered, which originated from the region of the temporalis muscles bilaterally; this was controlled with the electric cautery. The periosteum was then incised, and separated to the medial attachments of the temporalis muscles; the separation was continued 2-3 mm. beyond these attachments, along the line of attachment. At this point, considerable bleeding from cerebral emissary veins passing through the parietal bone was encountered. Homeostasis was achieved in this region by the use of small pledgets of gelfoam sponge, instead of the electrocautery, because of possible central nervous system damage from application of the cautery to the parietal bone for any length of time.

Four holes were drilled with the apparatus described above: Two holes were drilled immediately posterior to the fronto-parietal suture, 0.75 centimeters from the midline on each side; two more were drilled just anterior to the parieto-occipital suture the same distance from the midline

on either side.

A jeweler's screwdriver was provided with a spring clip to hold the segment of hypodermic tubing, to prevent entanglement of the electrode wire during insertion of the screw. The screws, with the wire and hypodermic tubing attached, were then threaded into the four holes previously drilled. Complete hemostasis of the entire area was then achieved with Gelfoam and electrocautery. All Gelfoam was then removed, and the entire bony operative site was scraped free of clotted blood and serum with a scapel blade. The area was then sponged with dry gauze, and further dried under a jet of compressed air for seven to ten minutes. The four segments of hypodermic tubing were then situated in an upright position a sufficient distance apart so that connectors to the recording apparatus could be attached to all four tubing segments without cross-interference. The entire operative site was then filled to a depth of approximately 0.5 centimeters with resinous dental cement, which was then allowed to dry. The skin around the operative site was then trimmed as necessary. Occasionally, a single 6-0 silk suture was placed to facilitate closure of the rear margin of the incision. After the cement had rendered the protruding segments of hypodermic tubing immobile, the animal was removed from the apparatus and returned to his cage.

III. Recording

The animal was kept in his cage throughout the recording procedure. Connectors between the animal's skull electrodes and the recording apparatus were fashioned by soldering a length of flexible insultated lead wire to a jeweler's clutch (usually used for retaining brooch pins, etc.). The four connecting wires were threaded through the galvanized mesh which served as the roof of the cage, and were attached to the animal. Each of the lead

wires was anchored with elastic bands, so that a small amount of tension was continuously maintained on the connecting wire, and slack in this wire which would result from the animals' movements would be effectively taken up. The entire cage was placed in a box whose sides were constructed of aluminum for shielding purposes. The EEG signal was conducted out of the box to the recording apparatus through shielded cable, which, together with the box itself, was grounded. During any individual recording procedure, it was not found necessary to anesthetize or otherwise restrain the animal in any manner, except for the boundaries imposed by the cage itself, and the minimal restraint imposed by the electrode connecting wires.

Bipolar EEG tracings were obtained with the Grass 8-channel electroencephalograph, model III-D. Because of the considerable movement artefact which occurred, it was found helpful to monitor the tracing with an osccilloscope connected to the second stage of the EEG power amplifier.

IV. Photic Stimulation

It was felt that interpretation of EEG recordings would be facilitated by the introduction of a suitable quantifiable parameter, and that the index of response of the animal to photic stimulation would fulfill the criteria for such a parameter.(9) Accordingly, the stroboscopic unit of a standard EEG photic stimulator was placed over the wiremesh roof of the animal's cage. The frequency of stimulation was governed by a trigger pulse which emanated from "synch" output of a Hewlett-Packard Low Frequency Function generator, Model 202A, which was accurate to plus or minus 2% of the stimulating frequency. The frequency of stimulation used varied between two and twenty-three cycles per second; tracings of photic responses were generally obtained at three particular frequencies: 3.75, 6.5, and 11.5 cycles per second. A self-generating selenium photocell was placed just

outside the animal's cage to detect the photic stimulus, and this was connected to the input amplifier of one of the EEG channels.

It can be readily seen that this arrangement does not constitute that used for usual photic stimulation experiments, since the animal is not expected to be gazing at the photic flasher unit in a continuous fashion throughout the duration of stimulation. However, the walls of the animal's cage reflected a considerable amount of the light introduced through the roof; thus, the photic stimulus consisted of a rapid increase and decrease in the level of illumination of the animal's entire cage. It is granted, however, that this mode of stimulation introduced another variable into the measurement of photic responses, i.e., the level of light striking the animal's eye as the position of his head was changed during the period of stimulation.

V. EEG Analysis

(A) Mechanical Analysis - An Edin model Electroencephalograph Frequency Spectrum Analyser was connected to the second stage of the power amplifier of each EEG channel. Before each recording, the analyser was calibrated with a signal of known frequency and peak-to-peak voltage, which was obtained from the low frequency function generator. Two modes of operation of the frequency analyser were utilized: In the first mode, a rotating scanner switch in the frequency analyser fed the amplified EEG signal into a sequence of sharply-tuned bandpass filter circuits. The center frequency of the filter networks had been preset, and varied between 0.5 and 45 cycles per second in progressively larger increments, the increment at the lower end of the frequency spectrum being 0.5 cycles per second between successive filter bands, and approximately 7 cycles per second at the upper end of the frequency range. The component of the EEG signal which had passed through

a particular filtering circuit was then fed into a diode pulser and a storage capacitor. The voltage accumulated across the condenser over a ten-second period was converted into a step-function excursion from the baseline by an output amplifier and pen recorder. The tracing obtained by this method was in the form of a bar histogram of voltage-seconds for each filter frequency. In the second mode of operation utilized for analysis, the scanner switch was set at a predetermined frequency (usually 3.75, 6.5, or 11.5 cycles per second) and the animal was stimulated at the identical frequency. The output of the filter network was shunted across the diode pulser-condenser system directly to the output amplifier. The tracing obtained was a continuous line whose height above the baseline was a function of the amplitude of the filtered frequency component of the EEG signal.

Another electrical method of displaying the various frequency components of the electroencephalogram simultaneously was attempted. A low-frequency sawtooth voltage (i.e., sweep voltage) was impressed across the x-axis terminals of a conventional cathode-ray oscilloscope, and a high frequency sweep voltage was similarly impressed across the y-axis input terminals. The output voltage from the second stage of an EEG channel power amplifier was connected to the z-axis (beam intensity modulation) of the oscilloscope through a condenser and triode voltage amplifier. In the absence of an EEG signal, the pattern observed upon the oscilloscope screen consisted of closely spaced vertical lines. When the EEG signal was fed into the z-axis amplifier, new patterns were superimposed upon that previously seen, which consisted of series of crisscrossing parallel lines of different thickness and orientations. The thicknesses of the lines seen could be shown to be function of the waveshape of successive EEG waves obtained on the tracing, while the orientation could be shown to be function of the

frequency component.

(B) Analysis by Visual Correlation - this was performed as follows:

(1) The number of low voltage, fast waves in each segment of EEG tracing representing one second was counted over a period of fifty consecutive seconds. For purposes of tabulation, one "wave" was defined as that portion of the tracing which was included between three successive points along the tracing where the first derivative of the wave function equalled zero, and the second derivative of the point included between the other two was opposite in sign (and usually negative) with respect to the other two points. The arithmetic mean of the frequencies in cycles per second for the fifty second period was determined, together with the standard deviation.

(2) In measuring the slow wave activity of the rat EEG, a "wave" was defined as an excursion of the tracing from the baseline which subsequently returned at least two-thirds of the way to the baseline. The frequency in cycles per second was again tabulated over a fifty second period, and the results treated as in the preceding paragraph. However, since slow wave activity was not present in many of the one-second time periods tabulated, the arithmetic mean was calculated only from those EEG segments exhibiting this activity, while the proportion of the total time period measured during which slow wave activity was present was estimated and expressed as a percentage.

(3) Photic responses were treated in approximately the same manner as those above. For reasons outlined in the DISCUSSION, the parameters obtained were not quantifiable as had originally been expected.

EXPERIMENTAL PROCEDURE

Seven animals were chosen for an experimental preliminary study of EEG responses to chronic morphine administration. These animals had been implanted with skull electrodes by the techniques previously described a minimum of six months before the experimental period was begun. These animals were of the Charles River Strain, and their weights ranged from 350-500 grams at the beginning of the experimental period. Morphine administration was initiated by the intraperitoneal injection of 12.5 mg./kg. of morphine sulfate which had been dissolved in normal saline. The injections were repeated approximately every twelve hours. After a total of six doses, the injected dose was doubled, and six injections at the new dosage level were administered at twelve-hour intervals. The dosage was increased geometrically in this manner, until the animal was receiving approximately 100 mg./kg. per injection, or a total of 200 mg./kg. per day. When the dosage level was reached, the dose was no longer increased, but was maintained at this level. At this point, the animal was considered to be in a state of "stabilized addiction." Five of the experimental animals were addicted to morphine in this manner. The other two animals were injected at twelve-hour intervals in a manner similar to the others; however, these animals received only intraperitoneal normal saline. The period of chronic administration of either morphine or saline lasted two to three weeks. At no time did the injected volume exceed two ml. per dose.

After the period of stabilized chronic administration with either morphine or saline had lasted three to seven days, all injections were stopped, and the animal abruptly withdrawn from the drug.

EEG tracings were obtained at the following times: A minimum of two control tracings were obtained before the period of chronic administration; a minimum of one tracing was obtained during the first two days of the chronic administration period; a minimum of one tracing during the period of "stabilized addiction"; and a minimum of one tracing five to eight days after abrupt withdrawal of the injected agent. Each tracing obtained usually consisted of a resting electroencephalogram obtained over a period of two minutes or longer, and a tracing of the animal's responses to at least one minute of photic stimulation at three different frequencies, usually 3.75, 6.5, and 11.5 cycles per second.

Immediately after the last EEG tracing was obtained from each animal, the animal was injected with 50 mg. of morphine, approximating the dose he received during the "stabilized addiction" period, to determine if tolerance to the drug had indeed diminished.

Because of the excessive amount of handling the experimental animals were subjected to, and for fear of the resultant dislodgement of the skull electrodes assembly, it was found necessary to obtund the animals with ether vapor immediately prior to each injection. The animal was placed in a transparent plastic container, throughout which ether vapor had been allowed to diffuse from a moist cotton pledget. As soon as the animal exhibited unsteadiness of gait, he was removed from the container and the injection was performed.

I. Effects of Morphine and Saline Administration

(A) Acute effects of administration of morphine. Administration of 12.5 mg./kg. of morphine to the experimental animals was unattended by signs of hypoventilation or drowsiness. No marked increase or decrease of the animal's activity or excitability was noted. No attempt was made in this series of experiments to evaluate the animals' performances in test situations, or to evaluate other physiologic parameters.

The administration of large doses (100 mg./kg.) acutely, e.g., in the unaddicted animal or in the addicted animals five to seven days after withdrawal, was accompanied by visible physiological and behavioral changes. These consisted of (1) marked, generalized increase of muscle tone, so that the animal became quite stiff and assumed an appearance resembling that of rigor mortis in the dead animal; (2) posterior extension and extreme stiffness of the tail, so that it was possible to lift the entire hind end of the animal by using the tail as a lever, as if it were a stiff rod rather than flexible; (3) extreme unresponsiveness to painful stimuli, such as pinching of the hind paws with a pair of forceps; (4) no loss of consciousness, and no demonstrable hypoventilation.

These effects were consistently observed and were thought to be a reliable index of the animals' sensitivity to morphine.

(B) Chronic Effects. After two to three days of chronic morphine administration, three of the five experimentally addicted animals developed a foul-smelling, watery diarrhea. This disappeared within three days. No other consistent effects were observed as a result of chronic morphine administration, with the dosage schedule used. However, it is notable that

during the period of stabilized administration, these animals did not exhibit the syndrome described in the preceding paragraph, even after receiving as much as 50 mg. of morphine twice daily. This was assumed to indicate that tolerance to the drug had developed in these animals.

(C) Effects of Withdrawal. No behavioral or physiologic effects which could be attributed to withdrawal of morphine or saline were noted. In particular, there were no deaths, evidences of hyperactivity or hyperexcitability, or seizures noted.

II. Characteristics of Control and Experimental Electroencephalograms

(A) Predominant EEG Patterns. By the methods outlined in the preceding section, tracings obtained from the unanesthetized, unrestrained animal consisted of the following:

(1) Thirty to sixty cycle fast activity, the voltage of which varied between approximately twenty-five and 125 microvolts. The frequency of this activity generally tended to be four to five cycles-per-second lower in the frontal and occipital leads, than in the parietal leads. The frequency of the fact activity tended to remain constant during any one recording session, usually not varying more than three cycles per second. (See illustration 1.)

(2) Slow waves, whose frequency range was two to nine cycles-per-second, with a voltage range of 100-225 microvolts, was present about seventy percent of the time during which recordings were obtained. Rarely, high voltage bursts of extremely regular eight to ten cycle-per-second waves were noted to occur spontaneously in all leads, but predominantly in the parietal leads. (See illustrations 2, 3.) The slow activity generally tended toward synchronization in the parietal leads bilaterally.

(B) Resting Patterns of Addicted Animals. No difference in resting EEG characteristics could be demonstrated in any animal between those records obtained during the period of chronic administrations, and the periods before and after such administration. These tracings possessed characteristics similar to those described above.

(C) Response to Photic Stimulation. The response obtained on the EEG tracing to photic stimulation was quite variable. The responses obtained included:

(1) No response - the EEG remained unaltered from the resting pattern, and no correlation between photic stimulation and the tracing obtained could be demonstrated. (See illustration 4.)

(2) Minimal response - this consisted of irregular bursts or trains of medium voltage (100-150 microvolts) waves, whose frequency tended to approximate that of photic stimulation. (See illustration 5.)

(3) Moderate response - this consisted of the minimal response described above, together with low voltage (50-100 microvolts) narrow spikes, which appeared synchronously with photic stimulation, or occasionally after a 0.025-0.05 second delay. (See illustration 6.)

(4) Moderate driving response - this was characterized by long bursts of spike-wave formations, the spike being coincident with the photic stimulus, or appearing with every alternating photic stimulus (driving at one-half frequency), with occasionally a spike latency similar to that described above. (See illustration 7.)

(5) Marked driving response - this consisted of high voltage (200-300 microvolts) spikes, synchronous with the photic stimulus. (See illustration 8.)

In general, photic responses were observed in all leads examined; they

tended to be most marked in the parietal leads. Spike formations showed either an upward or downward deflection in a variable manner.

The EEG Frequency Spectrum Analyser was not found to be helpful in quantifying the degree of response to photic stimulus. The Fourier component of the EEG at the frequency of photic stimulation did not appear to correlate with the intensity of photic response in a predictable manner.

(D) Photic Responses in Addicted Animals. Again, no difference was demonstrated between the intensity of photic response before and after chronic drug administration.

DISCUSSION

The intracellular locus of action of morphine has been thought to reside in one or more of the enzymes involved in the oxidation of glucose.(5) The profound nervous system effects of morphine and other narcotics would be in accordance with this hypothesis, because of the known sensitivity of the central nervous system to glucose deprivation. Effects which are exerted by a drug on an enzyme system, where the amount of enzyme synthesized per unit time is low, will tend to be tachyphylactic in nature.(10) Further extending this hypothesis, we can assume that tolerance to the effects of morphine is due to an increased enzyme synthesis rate in order to compensate for the acute effects of the drug. As a corollary, the symptoms due to abrupt withdrawal of the addicting agent would be explainable on the basis of the presence of an excess amount of enzyme and a consequent overutilization of glucose substrate. This hypothesis remains to be verified; however, indirect evidence supporting it does exist. The symptoms accompanying morphine addiction in rats have been thought to be ameliorated by the administration of cocarboxylase.(5)

Other effects which accompany the administration of morphine, even though they may not be occurring at the primary site of action of the drug, may be partly responsible for the syndrome occurring in narcotic addiction and withdrawal. It has been found by direct determinations of adrenal venous blood in the rat that single doses of morphine are capable of increase 17-hydroxycorticoid output from the adrenal 8-40 times resting values within ten minutes of administration of the drug.(11) Though nonspecific stress may also increase resting serum steroid levels within comparable periods, the former effect is much more specific for morphine.

The locus of action of morphine in producing this effect is thought to be the pituitary, because chronic administration of morphine to rats is followed by hypertrophy of the adrenal,[12] which does not occur in hypophysectomized animals.[13] The chronic effects of morphine administration on the adrenal are most probably unrelated to the effects. Urinary 17-hydroxycorticoids are diminished in chronic morphinism in the rat.[14] Morphine has further been noted to depress the diurnal increases in serum hydroxycortisone levels in human subjects, as well as to supress the increase normally produced by a standardized stress. However, the adrenals remain sensitive to ACTH stimulation after morphine administration.[15]

Changes in central nervous system tissue as a result of stabilized addiction may in part be due to a deficit of adrenal cortical hormone, or to ACTH,[16] or to changes in brain oxygen consumption secondary to hypoadrenocorticism.[17] ACTH alone has been noted to alter the EEG in one patient in whom adrenal activity had been suppressed by large doses of steroids.[18] It can be assumed, then that the proportion of nervous system changes during chronic morphinism that is contributed by changes in serum steroid level will be the sum of the acute and chronic effects of the drug, these perhaps on occasion tending to antagonize each other. This would also hold true if we wish to ascribe the changes due to the addiction state to altered serum ionic or intracellular concentrations; a relationship between EEG changes and serum ionic concentration has been established.[19]

In establishing criteria for addiction to morphine in lower mammals one is restricted by the difficulties involved in setting up experiments to demonstrate dependence and habituation in these animals, which usually assume prominent roles in human drug addiction. Tolerance to certain effects

of morphine, particularly the analgesic effect of a test dose of morphine, has been demonstrated in the rat.[20] Withdrawal symptoms in rats have not been as objectively characterized.

The syndrome observed in the present experimental series after acute administration of large doses of morphine has not been observed in the rat. However, a strikingly similar phenomenon (hyperextension of the tail) has been observed in the mouse after administration of morphine, and is the basis for the Straub "Maussesschwanzreaktion" for the detection of morphine.[21] After injection of small amounts of morphine, the tail of the animal becomes extremely rigid, and extends straight upward. This reaction has been presumed to occur as a result of reflex contraction of the anal sphincter. The corresponding syndrome in the rat may prove to be a reliable index of lack of tolerance to large doses of morphine.

The method described in a previous section for electrode implantation and EEG recording was modified from that of Hoagland.[22] Another modification of this method has previously been used in this laboratory.[23] The use of the method as herein described possesses many advantages, viz., (a) the entire implantation procedure can be performed in thirty minutes; (b) the method does not involve opening the skull of the animal; (c) electrodes will remain stably implanted for very long periods, as long as ten months; (d) muscle artefact is almost never seen on the tracing with this method; (e) artefact resulting from movement of the electrode connecting wires is easily distinguishable from the EEG tracing, and can be kept to a minimum by allowing the animal to become quiet in the absence of sensory stimulation for a short period of time.

The normal resting EEG of the rat as obtained by this method corresponds roughly to that obtained by other investigators,[24,25] and to that

obtained in this laboratory in an unrelated series of experiments involving EEG recording from unanesthetized but restrained animals.[23] It is felt that this recording method constitutes a valuable tool in the study of drug effects in the unanesthetized and unrestrained laboratory animal.

The responses to photic stimulation observed in these experiments corresponds to the responses obtained after similar stimulation in normal human adults.[26] However, the present series, responses were seen only at the frequency of stimulation and at integral submultiples of this frequency. Second and third harmonics were not noted, nor was high voltage irregular slow wave activity, nor other bursts of activity unrelated to stimulus frequency. The spike and wave discharges and the high voltage spikes which were observed are "abnormal responses" by human criteria. Thus photic stimulation by the techniques described might serve, with reservations, as a valuable adjunct in obtaining parameters from the EEG in these animals.

In human subjects, the frequency response to repetitive photic stimulation was found to be more easily analyzable when electronic frequency analysis was used as an aid in quantifying the responses. The present study has not confirmed this. Correlations of the Fourier components of the EEG tracing obtained with a commercial frequency analyser did not vary predictably with the intensity of the photic response. The reason for this is readily apparent when one considers that Fourier of a high-voltage, narrow spike recurring in an EEG tracing at, e.g., 3 cycles per second, will have a small Fourier component at 3 cycles per second. Thus, standard methods of electronic frequency analysis do not take into account the waveshape of the evoked response, which indeed may be its primary manifestation, in addition to the phenomenon of simply "following" the photic stimulus frequency.[27] The second method of frequency analysis described earlier did not prove

helpful because of instability of the circuitry as designed. However, it is felt that this method of visual display of the EEG signal will afford more information to the observer than any method previously described, once the errors resulting from non-linearities and instabilities in the system are corrected.

The EEG effects observed after ether anesthesia are not seen when the state of excitability of the cortex has returned to normal,[28] and this ensues within minutes after anesthesia is terminated. Small doses of morphine, such as those used in this experiment, have been shown to leave the EEG unaltered in cats for at least six minutes, although sedation has occurred. It has been shown that ether anesthesia has the propensity to augment activity from experimentally placed epileptogenic foci in rats;[29] this effect was also found to vary with the state of cortical excitability. Although bidaily etherization of the experimental animals in this study may have added an additional source of stress, it is doubtful that this affected the results to a significant degree.

The EEG effects observed after the acute administration of morphine have been noted to be variable. Gibbs, Bibbs, and Lennox[30] noted bursts of high-voltage 10 cycle-per-second waves. Gibbs and Maltby[31] noted a decrease in the predominant frequencies comparable to that occurring during sleep. Andrews[32] also noted a sleep pattern in one subject, although the subject was awake. In two others,[33] no obvious effect on the cortical potentials was demonstrable. Cahen and Wikler[25] noted that small doses of morphine (under 20 mg./kg.) administered to rats had little effect on the electrical activity of the cortex, although sedation was clearly evident. Larger doses (20-50 mg./kg.) produced bursts of high voltage 3 cycle-per-second waves, followed by very high voltage 12 cycle-per-second

activity. No further changes in the pattern were seen unless the animal was given doses large enough to cause respiratory depression and anoxia, (200 mg./kg.) and in these cases the cortical potentials were restored by artificial respiration. These changes were not considered to be specific in any way for morphine, but were thought to be analogous to those observed during light or moderate barbiturate narcosis. Andrews, in a study of 50 morphine addicts, found that stabilized addiction to morphine is characterized by significantly elevated alpha index in all leads.(32) In one patient who initially showed a predominance of fast frequencies, with an occipital alpha index below 10%, morphine was able to depress cortical excitation sufficiently to cause reversion of the rhythm to alpha frequency.(33)

The study herein contained cannot be regarded as possessing statistical significance; therefore Andrews' postulated association between narcotic addiction and EEG alterations is neither corroborated nor refuted. However, the observations made during this study tend to indicate that morphine addiction in the rat does not cause responses analogous to those in humans, when equivalent dosage ranges are used, perhaps because of the low sensitivity to the effects of morphine generally exhibited by these animals.

SUMMARY

1. New techniques for (a) chronic implantation of skull electrodes in the laboratory rat, (b) recording from unanesthetized, unrestrained animals, (c) photic stimulation, and (d) visual display of the EEG tracing are outlined and discussed.

2. Preliminary investigations of the EEG changes associated with morphine addiction in these animals indicate that the laboratory rat is probably not a suitable subject for such experiments, due to its inherent unsusceptibility to the effects of this drug.

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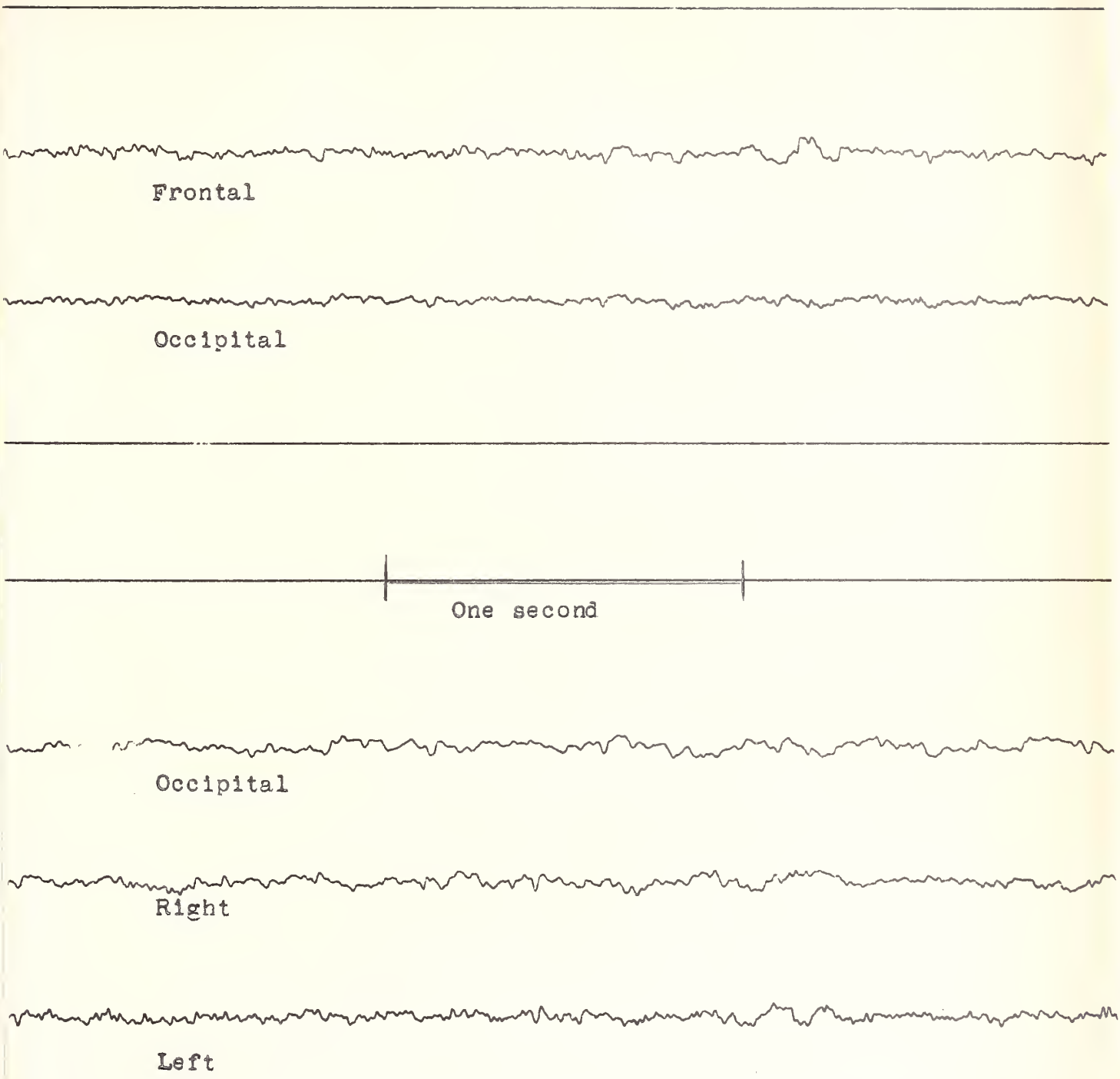

Illustration 1. Rat number 4, Feb. 25, 1961. Tracing shows minimal slow activity superimposed on low voltage fast activity.

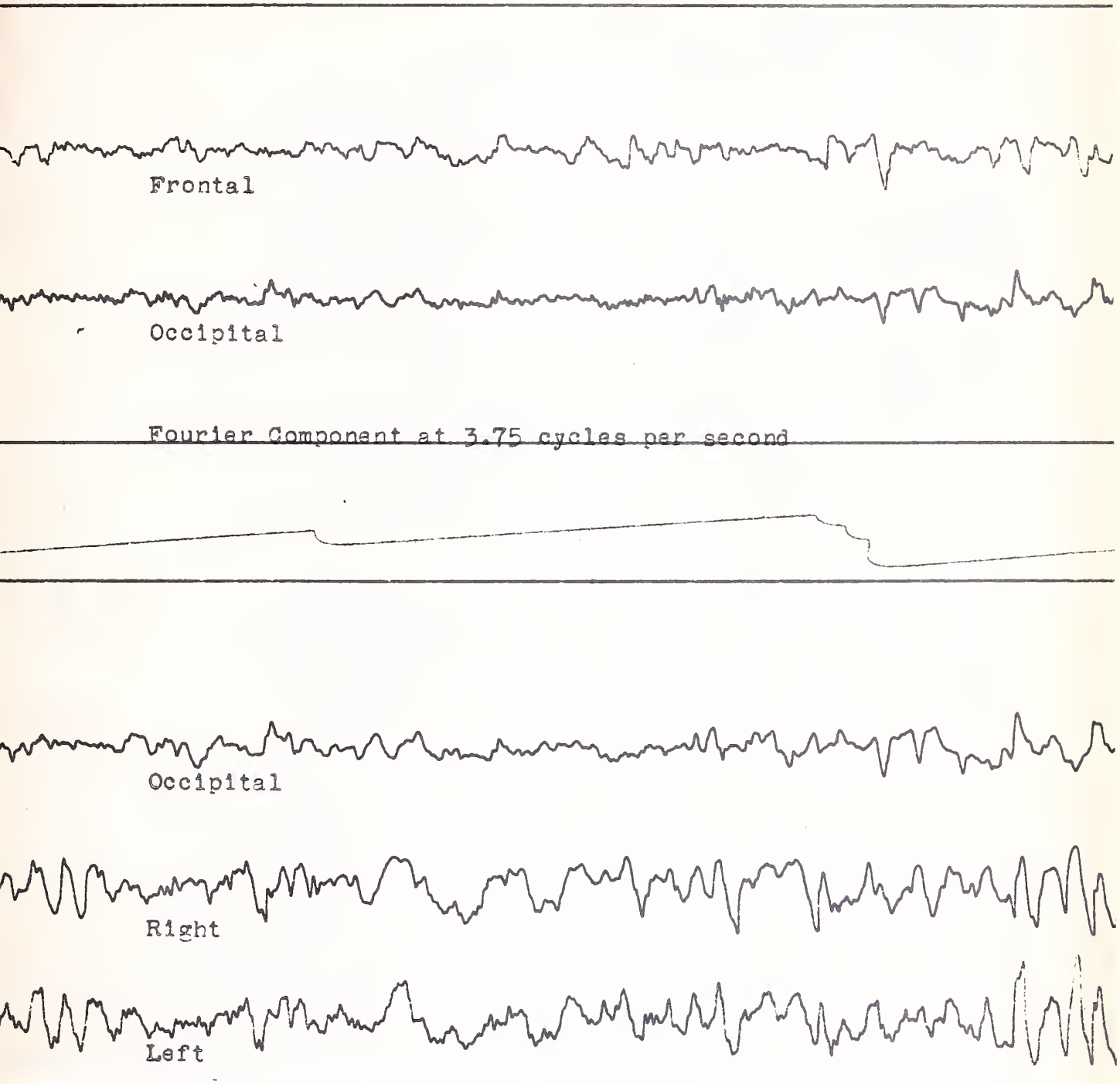

Illustration 2. Rat number 12, Feb. 1, 1961. Tracing shows moderate amount of slow wave activity superimposed upon low voltage fast activity.

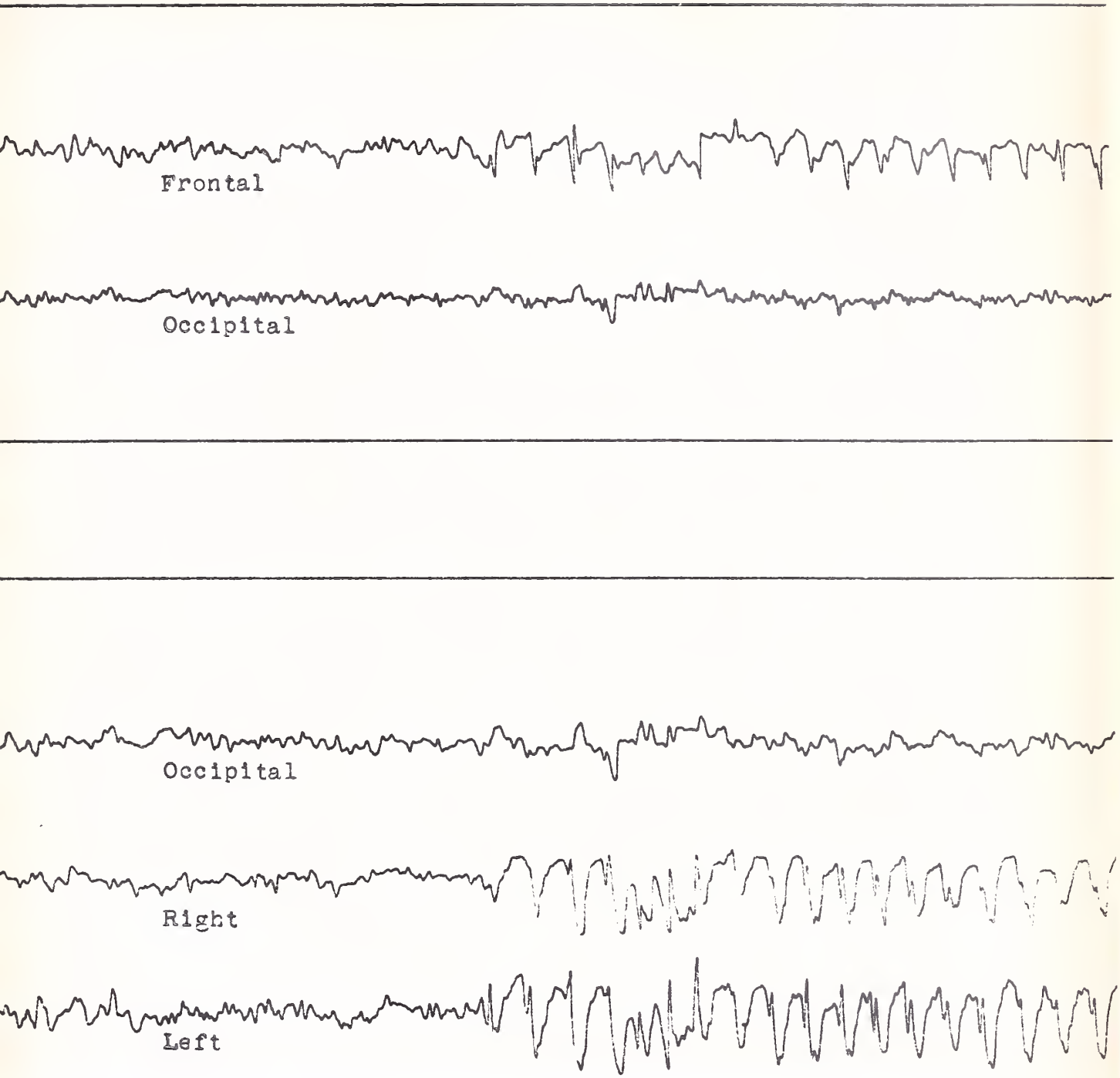

Illustration 3. Rat number 16, Feb. 28, 1961. Note spontaneous runs of high voltage 8-10 cycle-per-second waves.

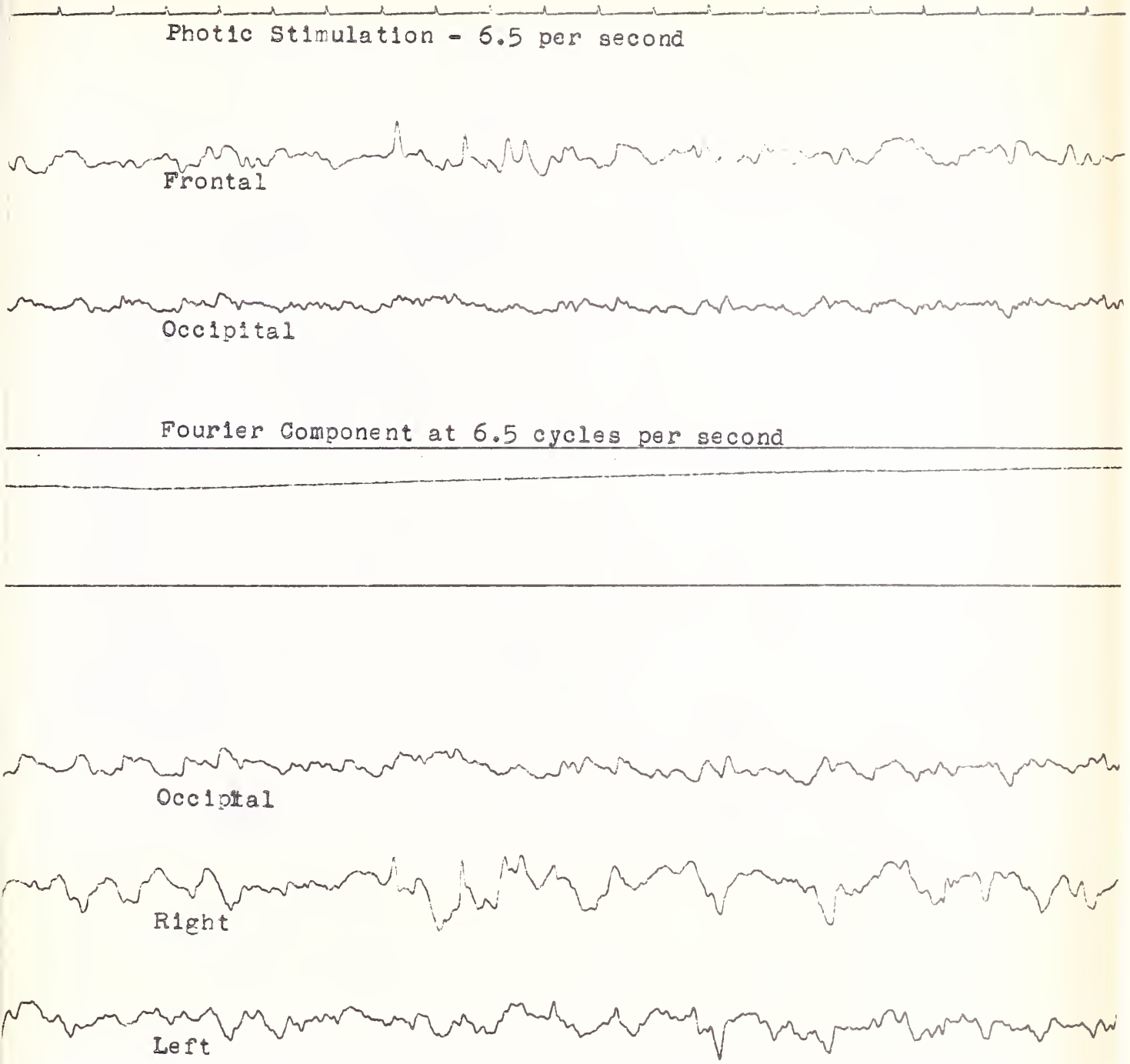

Illustration 4. Rat number 3, Jan. 22, 1961. There is no response to photic stimulation.

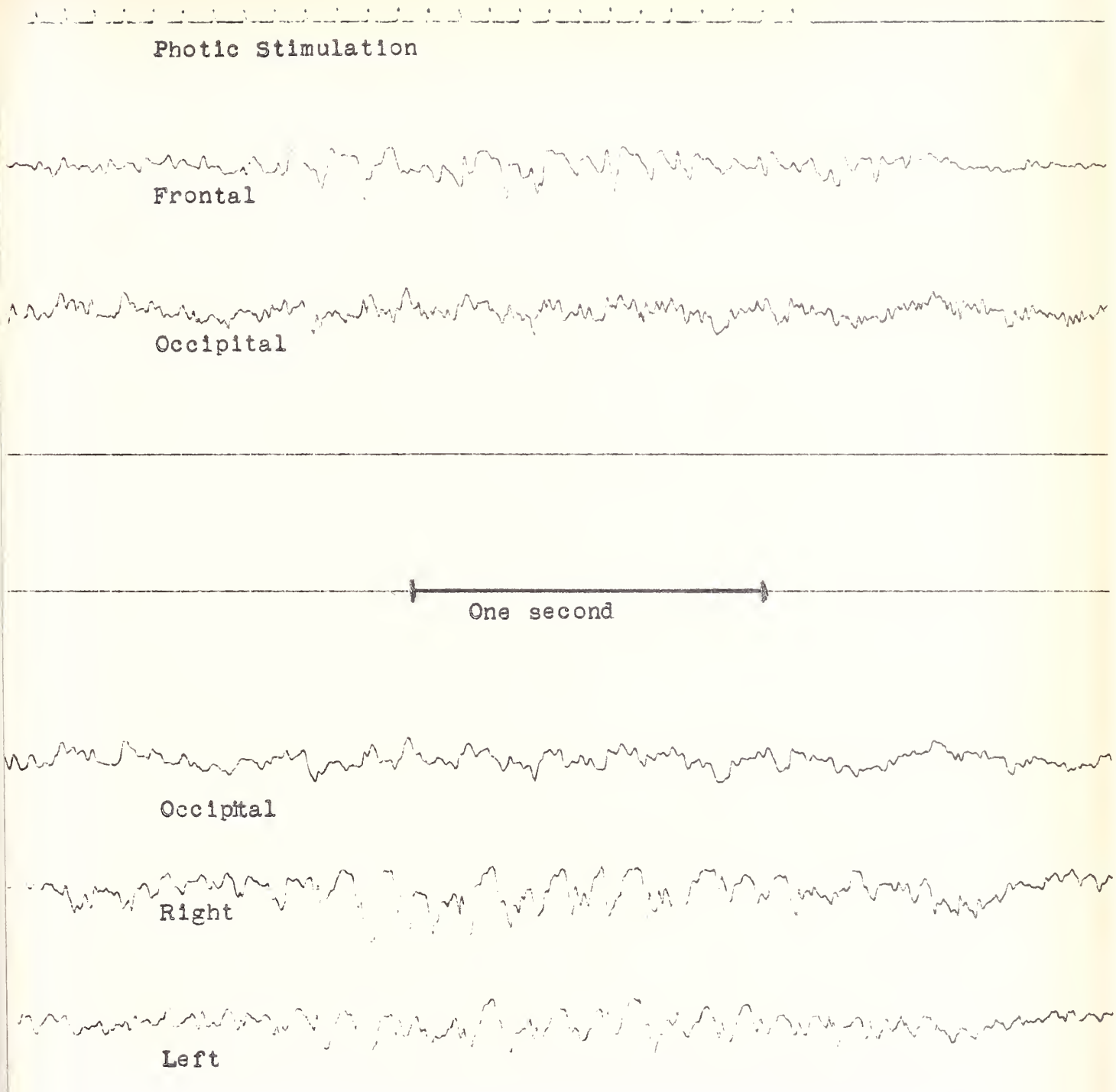

Illustration 5. Rat number 4, March 1, 1961. Minimal response to photic stimulation.

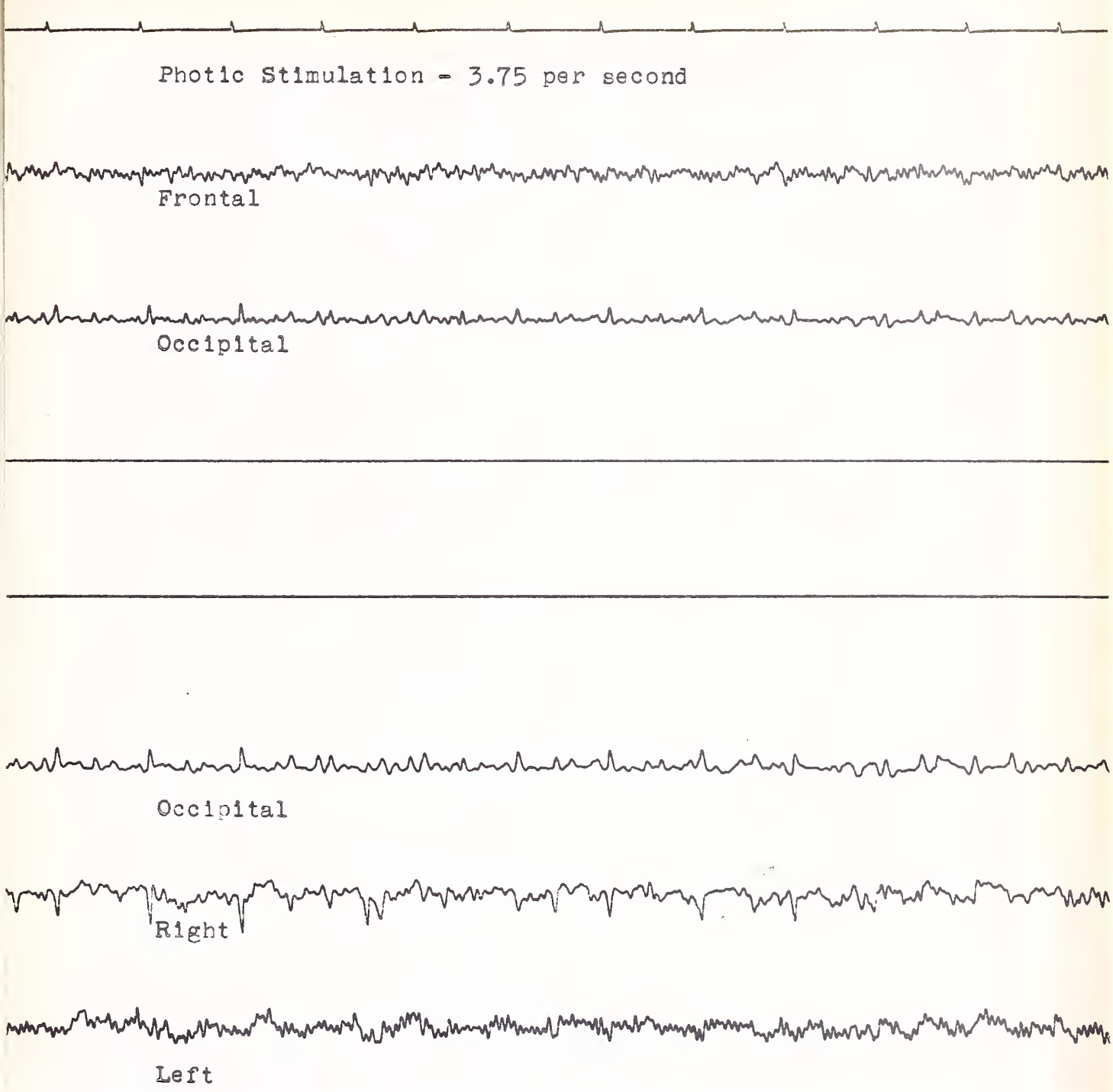

Illustration 6. Rat number 13, Feb. 10, 1961. Moderate response to photic stimulation (see text).

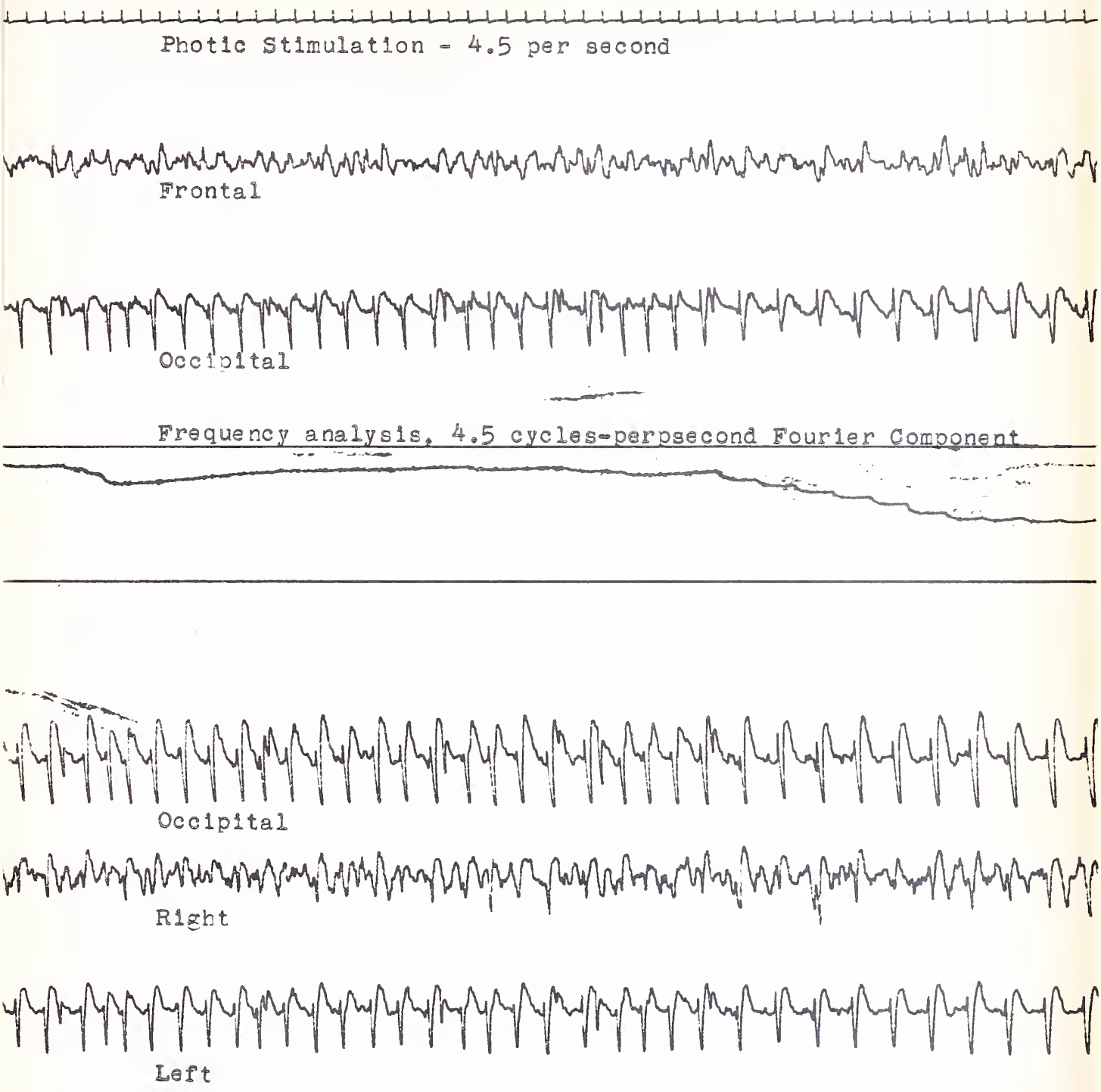

Illustration 7. Rat number 3, Jan. 23, 1961. Moderate driving response.

Note spike-and-wave formations.

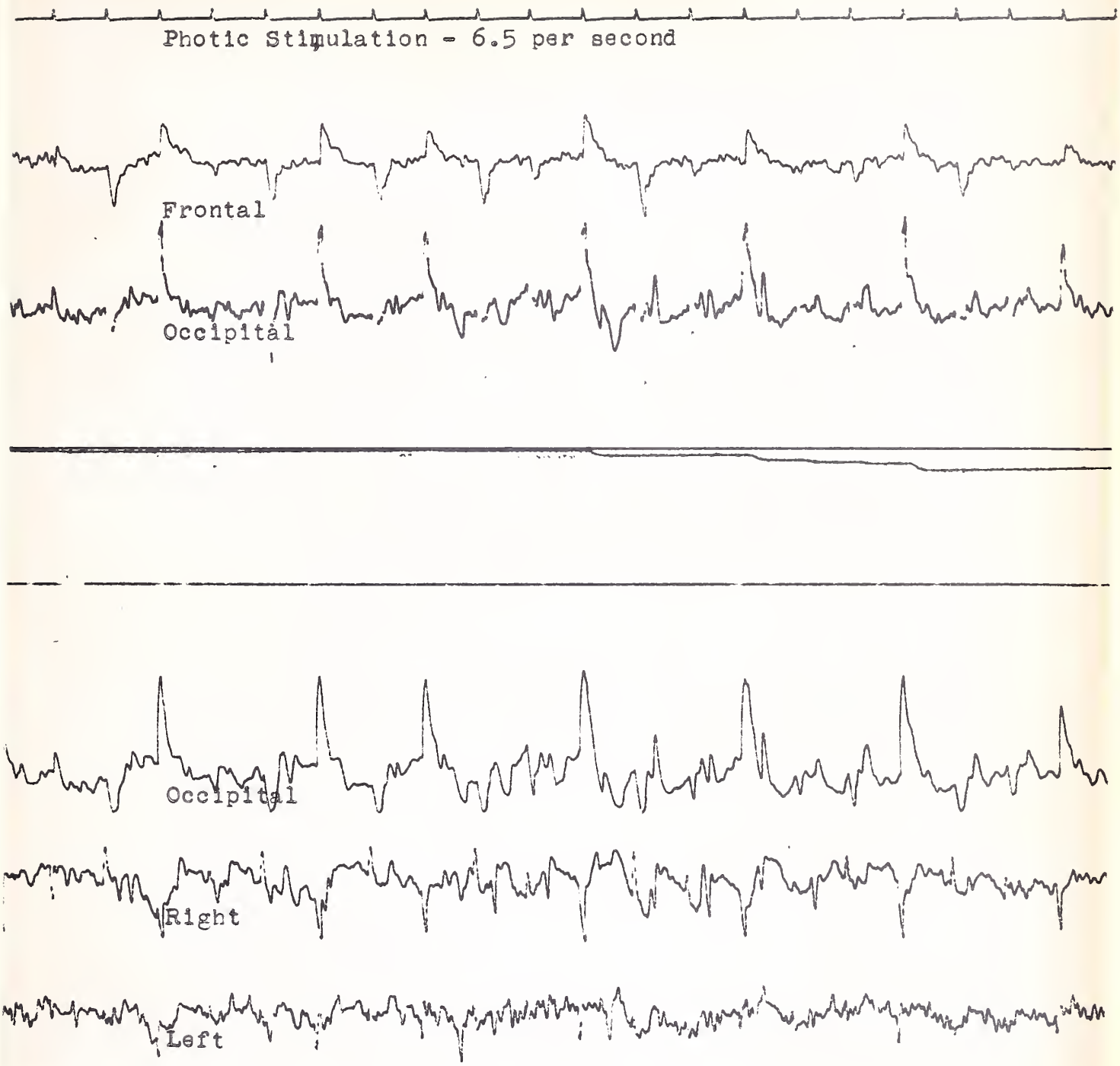

Illustration 8. Rat number 12, Feb. 3, 1961. Marked driving response.

Note high voltage spikes.

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